Remarks/Arguments

The present amendment amends claim 12, adds new claims 20-23, and cancels claims 13 and 15 without prejudice to future prosecution. The amendment to claim 12 changes "combining incubating" to "incubating". The amendment submitted November 12, 2004 inadvertently added "combining" without any underlying.

New claims 20 and 21 are intended to replace canceled claims 13 and 15. Claim 20 corresponds to claim 13. Claim 21 is dependent on claim 20, and corresponds to claim 15.

New claim 22 is along the same lines as claim 12, but does not indicate that NS5B is purified to apparent homogeneity. Claim 23 depends from claim 22 and indicates the method measures primer independent RNA-dependent RNA polymerase activity.

The previous office action rejected the claims as follows:

1. Claims 12-15, 17 and 18 were rejected based on obviousness-type double patenting in view of U.S. Patent No. 6,383,768; and
2. Claims 12, 14, 17 and 18 were rejected as allegedly obvious based on Tomei et al. (Journal of Virology 67(7): 4017-4026, July 1993).

There rejections are respectfully traversed.

Obviousness-Type Double Patenting

Enclosed is a terminal disclaimer addressing the obviousness-type double patenting rejection.

35 U.S.C. § 103 (Tomei et al.)

The present application successfully establishes that NS5B produced in an artificial expression system provides RNA-dependent RNA polymerase activity encoded by HCV. The application further demonstrates that NS5B can be successfully purified to apparent homogeneity and have sufficient activity to be used in a method for identifying a HCV RNA-dependent RNA polymerase inhibitor.

Tomei et al. fails to demonstrate that NS5B: (1) corresponds to an authentically produced HCV protein; (2) is responsible for producing RNA-dependent RNA polymerase activity; and

(3) can be successfully purified. It is respectfully submitted that the prior art fails to provide a reasonable expectation of success in modifying Tomei et al. to produce the claimed assay.

The deficiencies in Tomei et al. and the prior art are highlighted by publications describing uncertainties as to whether NS5B produced in an artificially expression system corresponds to an authentically produced HCV protein and publications illustrating additional considerations. Additional considerations include difficulties in obtaining active HCV RNA-dependent RNA polymerase activity, purifying HCV RNA-dependent RNA polymerase activity and long-felt need for a HCV RNA-dependent RNA polymerase assay.

Tomei et al

Tomei et al. identifies NS3 as a serine protease required for the processing of HCV polyprotein. Using a recombinant expression system, Tomei et al. found that NS3 was essential for cleaving HCV polyprotein to produce different proteins including a particular region designated "NS5B".

The rejection argues that Tomei et al. should be modified based on the following motivations: (1) motivation to incubate NS5B, ribonucleotide substrates and an RNA template to characterize the function and role of NS5B; (2) motivation to produce NS5B to determine whether proteolytic processing affects NS5B protein product; (3) motivation to vary the RNA templates and primers to characterize RNA-dependent RNA polymerase activity; (4) motivation to add ribonucleotide substrates and RNA template based on the suggestion in Tomei that NS5B may encode a RNA-dependent RNA polymerase; and (5) motivation to add potential target molecules to identify potential HCV therapeutics against HCV.

The rejection argues for a reasonable expectation of success based on Tomei et al. suggesting that the NS5B open reading frame encodes a RNA-dependent RNA polymerase, the high level of skill in the art, that the HCV genome is processed in a similar manner as flaviviruses and pestiviruses, and that the hydropathy profile of the HCV polyprotein is similar to that of the flavivirus.

HCV Polyprotein Processing

Prior to the present application it was not known whether HCV NS5B produced in recombinant expression systems such as that employed by Tomei et al. corresponded to a naturally produced product. **NS5B is not produced from an open reading frame** encoding the protein, but is produced from proteolytic processing of recombinantly expressed HCV polyprotein.

Motivations such as characterizing the function and role of NS5B and determining whether proteolytic processing affects NS5B reflect the uncertainties in the art. Such motivations amount to an invitation for studying HCV processing, and do not provide a reasonable expectation of success in obtaining a functional HCV RNA dependant RNA polymerase assay that could be used to screen for inhibitors. The high level of skill relates to the ability of the skilled artisan to perform certain activities and does not resolve the scientific uncertainties existing in the prior art.

The uncertainties concerning the relevance of recombinant HCV processing to authentic HCV processing is reflected in the differences between published results obtained from HCV infected liver cell versus recombinantly processed HCV polyprotein. The uncertainties are also noted in cautionary language used in publications concerning recombinant NS5B.

Tsutsumi et al. (Hepatology 19(2), 265-272, 1994) notes prior work using a recombinant system that produced NS5B and observed that HCV in infected liver cells did not produce a protein with approximately the same molecular weight as NS5B:

> Grakoui et al. (15) reported that two proteins were derived from the HCV-NS5 region: NS5A (58kD) and **C-terminal NS5B (66 to 68 kD)**, when a cDNA encompassing the long open reading frame was used in the vaccinia virus transient-expression assay. The NS5B protein was predicted to contain the RNA-dependent RNA polymerase activity on the basis of the presence of the characteristic Gly-Asp-Asp and surrounding conserved motifs. Although bacterially expressed HCV-NS5 peptide fragment was used for a part of NS5B protein in this study, the molecular size of **HCV-NS5-related antigen detected in human liver was 86 kD** and thus slightly larger than that of NS5B. This discrepancy may have resulted in different host cells, which were cultured mammalian cells in Grakoui's study (15) and were human liver cells in this study. Furthermore, we observed the products derived from native HCV, whereas Grakoui et al., (15) observed the polypeptide expressed from HCV cDNA in vaccinia virus. [Emphasis added.]

(Tsutsumi et al. starting at page 269, first column, second paragraph to page 270, first column.)

The magnitude of the different molecular weights for the infected human liver and recombinantly processed HCV NS5 regions point to different proteins and not minor variations. The 86 kD molecular weight noted by Tsutsumi et al. is 21 kD more than the Tomei et al. NS5B weight of 65 kD and 18 kD more than the upper 68 kD attributed to Grakoui et al. Assuming the average molecule weight of a natural amino acid is 137, 18 kD provides for over 100 amino acids.[1]

Both Tomei et al. and Grakoui et al. (Journal of Virology 67(3), 1385-1395, 1993) point out that recombinantly produced NS5B may not correspond to a naturally produced product. According to Tomei et al.:

> It is clear, however, that the results obtained with this transient expression system may **not faithfully reproduce the proteolytic events which take place during HCV infection**. It is possible that the level of protein expression obtained in this system may be much higher than normal, affecting important equilibria between precursors and proteases, which in turn may regulate HCV replication and protein synthesis. [Emphasis added.]

(Tomei et al., at page 4025, first column, this paragraph.) Similarly, Grakoui et al. notes in its concluding paragraph:

> The experiments reported here have given us a preliminary picture of HCV polyprotein organization and processing. However, **this view is far from complete, and additional studies are needed to define polyprotein cleavage sites** and the responsible proteinases and to verify that the products observed in these expression studies are similar to those produced in authentic HCV infections. Such information should prove valuable for expression and characterization of HCV-encoded enzymes as potential targets for antiviral therapy and will allow future studies . . . [Emphasis added.]

(Grakoui et al. at page 1393, first column, third paragraph.)

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[1] Molecular weights for naturally occurring amino acids vary from 75.07 to 204.3 (CRC Handbook of Chemistry and Physics 81st Edition, David Lide Editor, 7.1 CRC Press, 2000-2001.) An average molecular weight of 137 is based on the individual weights of the amino acids and does not take into account the amino acid frequency in a protein.

The present application demonstrates that NS5B provides for an HCV RNA-dependent RNA polymerase. Neither Tomei et al., Grakoui et al., or Tsutsumi et al. provide results demonstrating that an observed protein provides HCV RNA-dependent RNA polymerase activity. Thus, the present application itself, not the prior art, resolves the scientific uncertainties concerning the activity of NS5B and may call in to question the assertions provided by Tsutsumi et al.

Additional Considerations

Additional considerations pointing out the inventive nature of the pending claims include apparent failure and difficulty encountered by others and long-felt need. Apparent failure and difficulty encountered by others is evident based Chung et al. (Hepatology, 16(4), 1992) and WO 97/12033. The long-felt need is apparent based on the importance of HCV and the time delay between published speculation concerning the HCV RNA-dependent RNA polymerase and the present application.

Chung et al. is an abstract mentioning attempts to obtain HCV RNA-dependent RNA polymerase from liver tissue. Chung et al. makes reference to activity obtained with partially purified extracts. Reference to only partially purified activity is consistent with failed attempts to obtain a purified product. Failed purification attempts are evident based on use of different chromatographic techniques and the desirability to obtain a purified enzyme for further study.

Chung et al. also directs the skilled artisan away from using recombinantly produced HCV protein by indicating the use of liver extracts. However, Chung et al. does not provide sufficient information to enable the skilled artisan to reproduce the assay or to verify the assay was successful. Important missing information includes how liver tissue samples were obtained and treated, what conditions were employed for the assay, and which conditions were employed for purification.

WO 97/12033 has a priority date of September 27, 1995, which is about four months after the priority date for the present application. According to WO 97/12033, in the Background on the Invention:

> The non-structural protein designated 5B (NS5B) has been shown to have an amino-terminal sequence SMSY (Ser-Met-Ser-Tyr). The NS5B region encodes a 68kd protein (p68) which contains an internal GDD (Gly-Asp-Asp) motif found in RNA-dependent RNA polymerases of other RNA viruses (Koonin, E. V. (1991) *J. Gen. Virol.* 72:2197-2206). **However, no polymerase activity has been detected for HCV p68.** In fact, the question has been raised that the 5B protein (p68) alone does not encode an active RNA-dependent RNA polymerase enzyme and that another subunit, possibly the NS5A gene product, is essential to catalytic activity. **Prior attempts by the inventors and others to express the NS5B coding region as a fusion protein, using existing expression systems that facilitate purification of the fusion product and specific cleavage have failed to yield any active polymerase.** [Emphasis added.]

(WO 97/12033, at page 2, line 25 to page 3, line 3.)

The long felt-need is evident based on the medical importance of HCV, the desirability to obtain an HCV RNA-dependent RNA polymerase assay to screen for anti-viral compounds, and the time difference between prior art speculations concerning HCV RNA-dependent RNA polymerase and applicant's priority application. Speculations concerning the HCV protein responsible for RNA-dependent RNA polymerase are noted at least as early as 1990. (Miller et al., (Proc. Natl. Acad. Sci. USA, March 1990, 87(6), 2057-2061).) The present application has a priority date of May 25, 1995, which is more than five years after Miller et al. was published.

Accordingly the claims are in condition for allowance. Please charge deposit account 13-2755 for fees due in connection with this amendment. If any time extensions are needed for the timely filing of the present amendment, applicants petition for such extensions and authorize the charging of deposit account 13-2755 for the appropriate fees.

Respectfully submitted,

By _____

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